

Fumigation toxicity of eucalyptus oil and its active compound against dried fish pest, *Dermestes maculatus* Degeer (Coleoptera: Dermestidae)

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Abstracts: To assess the insecticidal activity of eucalyptus oil and α -pinene against dried fish pest *Dermestes maculatus*. Mortalities of its larvae and adults under different doses or treatment time were investigated by bioassay method. The results showed that the tested oil and its active ingredient (α -pinene) affected significantly ($P < 0.001$) on the mortality of both the larvae and adults of *D. maculatus*. Responses varied with compound, dose and exposure time. Over 90% mortality at 72 h after treatment was achieved with the eucalyptus oil against larvae at a dose of $32 \mu\text{L}/\text{cm}^3$. Eucalyptus oil at $32 \mu\text{L}/\text{cm}^3$, were highly toxic to adults of *D. maculatus* 72 h after treatment. α -Pinene also showed highly toxic effects on both larvae and adults of the beetles at the same exposure time and doses. It is also interesting to mention that the adults of *D. maculatus* were found to be more tolerant than larvae. In probit test eucalyptus oil was much more effective than α -pinene. These naturally occurring materials could be useful for managing populations of *D. maculatus*.

Key words: Eucalyptus oil; α -pinene; fumigation; adults; larvae; dried fish

1 INTRODUCTION

Freshwater fish is a very important source of animal proteins in the world. Currently, approximately 16 percent of animal proteins consumed by the world's population are derived from fish, and over one billion people worldwide depend on fish as their main source of animal proteins (FAO, 2000). Worldwide consumption of fish as food has risen from 40 million tons in 1970 to 86 million tons in 1998 (FAO, 2002). Dried fish often is an alternative to fresh fish in many other places, it is highly nutritious and economically important. Dried fish is increasingly used to correct protein deficiency in normal diet (Laureti, 1998). The high susceptibility and deterioration of smoked fish products put losses arising from microbial and insect pest infestation in the tropics at between 20% and 50% during storage time (Lale and Sastawa, 1996). Properly dried fish will remain edible for many weeks, but it is very prone to infestation by insects including *Dermestes ater* Deg. and *D.*

maculatus Deg. (Coleoptera, Dermestidae), *Necrobia rufipes* Deg. (Coleoptera, Cleridae), and *Chrysomya marginalis* Wied. (Diptera, Calliphoridae) (Odeyemi *et al.*, 2000). All these insects are generally associated with dried fish especially during storage, transportation and marketing (Don-Pedro, 1989). *D. maculatus* is a major insect pest of dried fish in tropical and sub tropical countries. *D. maculatus* attacks dried animal flesh, bones, hide, horn and feathers (Hinton, 1945). It also destroys many other stored products including dried meat, dried blood and leather cheese (Osuji, 1973). Insecticides used or recommended for the protection of dried fish, hides and skins include naphthalene, sodium arsenite, sodium silicofluoride, lindane, dieldrin, DDT, carbaryl and malathion (Kritzing, 1955; Shuttleworth and Gallway, 1961). Also larval stages of *Dermestes* spp. usually account for infestation of about 93% in dried fish (Osuji, 1973). Therefore, research on the use of natural pesticides for storage is on the increase because of their reduced toxicity to human (Yee and Toscano, 1998; Lee, 2005).

基金项目: 华中农业大学植物科技学院作物学博士后基金(2006BAZ09B04-06)

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收稿日期 Received: 2008-10-07; 接受日期 Accepted: 2008-12-16

Many plant extracts, essential oils, and their constituents manifest fumigant (Kim *et al.*, 2003; Jang *et al.*, 2005; Isman, 2006; Rajendran and Sriranjini, 2008) because they constitute a rich source of bioactive chemicals. Much effort has, therefore, been focused on plant-derived materials and potential sources of commercial insect-control agents.

This paper describes a laboratory study to assess the potential fumigant effects of eucalyptus oil and α -pinene on the larvae and adults of dried fish pest, *D. maculatus*.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Test insects: Stock cultures of strains of *D. maculatus* Deg. were obtained from the Institute of Insect Resources Laboratory, Huazhong Agricultural University, Wuhan, Central China, where they had been isolated from all kinds of insecticides for several years. Cultures of *D. maculatus* were established by adding about 50 young adult beetles to a mixture of fishmeal (400 g) and dried yeast (25 g) in a Kilner jar (2.7 L). A wad of wet cotton wool was placed at the bottom of the jar to encourage oviposition. After three weeks the parental adults were removed from the culture medium and the jars resealed with filter paper secured by paraffin wax. All insect cultures were kept at 28°C and 70% RH under constant red light. All bioassays were conducted under these same conditions of temperature, humidity and light.

2.1.2 Eucalyptus oil and active ingredients: Industrially extracted eucalyptus oil (99%) and α -pinene (99%) were obtained from Carl Roth GmbH, Berlin, Germany.

2.2 Bioassay

The susceptibility of *D. maculatus* larvae and adults to the fumigant action of eucalyptus oil and α -pinene was investigated according to the method of Kim and Ahn (2001) with some modifications. Doses used 0.5, 1, 2, 4, 8, 16 and 32 μL for oil and active ingredients, dissolved in 1 mL acetone and applied to a piece of filter paper (Whatman, 3.0 cm diameter) which was dried in air for 5 min and was placed in a glass bottle (1 L). In the control only 1 mL acetone was applied to filter paper (0). Twenty larvae and adults were kept in small glass tubes (40 mm \times 60 mm) with open ends covered with 40-mesh copper mesh and containing 5 g of dried fish. The tubes were hung at the

geometrical centre of the glass bottles, which were then sealed with air-tight lids. The insects were exposed and mortality counts at 6, 12, 24, 36, 48 and 72 h after treatment with the series of concentrations. All treatments were replicated three times. Insects were considered to be dead if appendages did not move when prodded with a camel's hair brush. Data were corrected for control mortality (Abbott, 1925), Corrected mortality = (Total mortality - Control mortality)/Total number of insect used \times 100. Whole experiment was held at $30 \pm 10^\circ\text{C}$, 70% - 80% relative humidity.

2.3 Statistical analysis

The dose-mortality response was analysed by probit analysis (Finney, 1971) using the maximum likelihood programme software. Data were analysed by using ANOVA with a factorial design (Minitab Inc.).

3 RESULTS

3.1 Effects of eucalyptus oil and α -pinene against *D. maculatus* larvae

The vapor-phase toxicity of plant derived compounds *viz.* eucalyptus oil and α -pinene on the mortality of mature larvae of dried fish pest *D. maculatus* was studied and showed significant ($P < 0.001$) effect. Responses varied according to dose and exposure time. At the highest dose of eucalyptus oil 32 $\mu\text{L}/\text{cm}^3$ and 72 h of exposure, 92% mortality ($F = 180.08$, $df = 7, 41$, $P < 0.001$) of the larvae was achieved; at the lowest dose (2 $\mu\text{L}/\text{cm}^3$) and 72 h of exposure, eucalyptus oil caused over 68% mortality ($F = 162.36$, $df = 7, 41$, $P < 0.001$) against larvae of *D. maculatus* (Fig. 1). α -Pinene caused over 90% mortality ($F = 162.36$, $df = 7, 41$, $P < 0.001$) against the larvae at the highest dose of 32 $\mu\text{L}/\text{cm}^3$ and 72 h of exposure (Fig. 2). *D. maculatus* larvae were more susceptible against eucalyptus oil and α -pinene than adults.

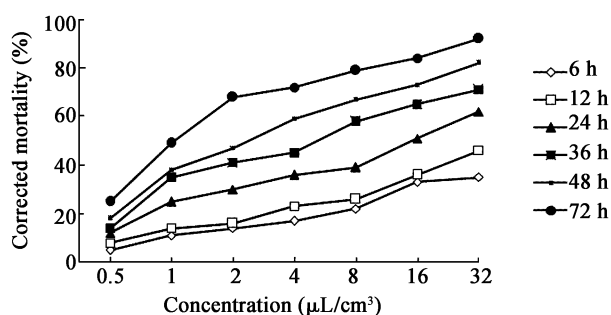


Fig. 1 Corrected mortality of *Dermestes maculatus* larvae fumigated with eucalyptus oil at different exposure time and concentrations

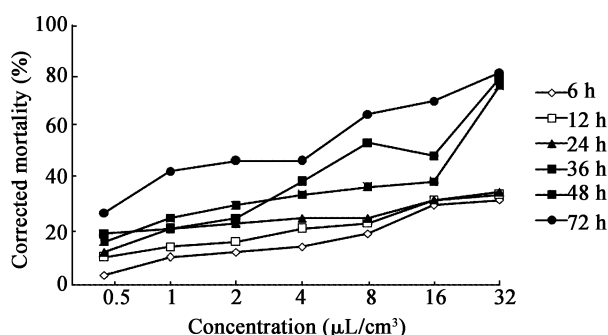


Fig. 2 Corrected mortality of *Dermestes maculatus* larvae fumigated with α -pinene at different exposure time and concentrations

3.2 Effects of eucalyptus oil and α -pinene against *D. maculatus* adults

The fumigant toxicity of eucalyptus oil and α -pinene on the mortality of *D. maculatus* adults was studied and showed significant ($P < 0.001$) effect. Responses varied according to dose and exposure time. Eucalyptus oil caused over 82% mortality ($F = 9.47$, $df = 7, 41$, $P < 0.001$) of the adults at $32 \mu\text{L}/\text{cm}^3$ and 72 h of exposure; while at the lowest dose ($2 \mu\text{L}/\text{cm}^3$) and 72 h of exposure eucalyptus oil caused over 48% mortality ($F = 180.08$, $df = 7, 41$, $P < 0.001$) against *D. maculatus* adults (Fig. 3). α -Pinene caused over 88% mortality ($F = 180.08$, $df = 7, 41$, $P < 0.001$) against the adults at the highest dose of $32 \mu\text{L}/\text{cm}^3$ and 72 h of exposure (Fig. 4). *D. maculatus* adults were more tolerant than larvae against the phytochemicals.

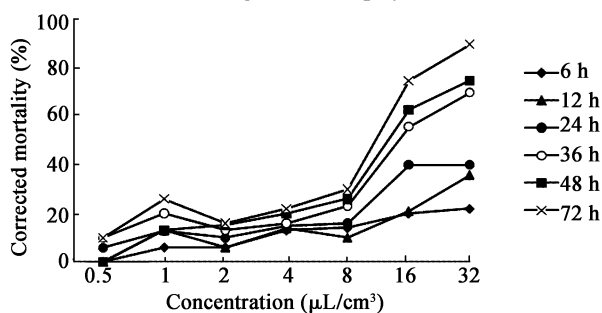


Fig. 3 Corrected mortality of *Dermestes maculatus* adults fumigated with eucalyptus oil at different exposure time and concentrations

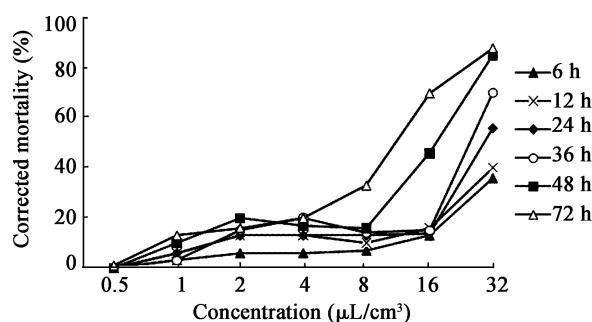


Fig. 4 Corrected mortality of *Dermestes maculatus* adults fumigated with α -pinene at different exposure time and concentrations

3.3 Probit results of eucalyptus oil against larvae and adults of *D. maculatus*

Probit mortality values of eucalyptus oil against larvae and adults of *D. maculatus* at different exposure time and doses are summarized in Table 1 & Fig. 5. LT_{50} values of eucalyptus oil were 75.21, 47.53, 56.63, 49.79, 47.28, 27.11 and 11.04 h against larvae, and 145.87, 115.74, 104.76, 90.15, 46.79, 42.05 and 16.98 h against adults at 0.5, 1, 2, 4, 8, 16 and $32 \mu\text{L}/\text{cm}^3$ doses respectively (Fig. 5). LD_{50} values for the eucalyptus oil against *D. maculatus* larvae were 20.72, 1.84 and $0.65 \mu\text{L}/\text{cm}^3$, and 186.13, 6.12 and $2.28 \mu\text{L}/\text{cm}^3$ against *D. maculatus* adults at 24, 48 and 72 h of exposure respectively (Table 1). The results varied significantly ($P < 0.001$) and there was no overlapping at 95% CL. The results showed that the larvae were more susceptible to phytochemicals.

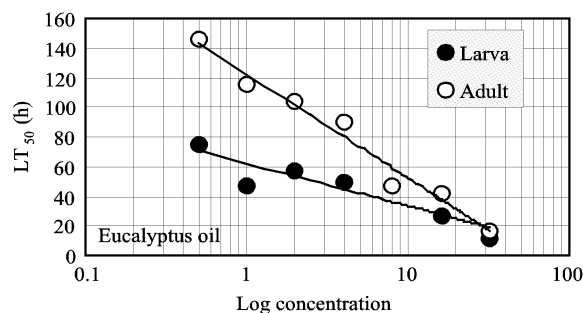


Fig. 5 Probit mortality for lethal time of *Dermestes maculatus* larvae and adults fumigated with eucalyptus oil at different concentrations

Table 1 Probit mortality for the eucalyptus oil against *Dermestes maculatus* fumigated at different time intervals

Developmental stage of insect	Exposure (h)	LC_{50} ($\mu\text{L}/\text{cm}^3$)	Lower - Upper (95% CL)	Slope \pm SE	χ^2	Probability
Larva	24	20.72	11.29 - 65.72	0.85 ± 0.18	9.50	0.91
	48	1.84	0.02 - 6.02	0.36 ± 0.15	9.51	0.91
	72	0.56	0.12 - 1.17	0.75 ± 0.17	3.59	0.39
Adult	24	186.13	37.99 - 499.7	0.42 ± 0.14	1.41	0.08
	48	6.12	3.81 - 10.80	0.72 ± 0.13	9.74	0.92
	72	2.28	1.20 - 3.71	0.69 ± 0.13	4.89	0.57

3.4 Probit results of α -pinene against larvae and adults of *D. maculatus*

LT₅₀ values of α -pinene were 278.38, 250.4, 126.36, 105.36, 59.52, 30.45 and 18.13 h against larvae, and 340.2, 262.1, 217.82, 157.36, 75.79, 45.32 and 44.79 h at 0.5, 1, 2, 4, 8, 16 and 32 $\mu\text{L}/\text{cm}^3$ doses respectively. LD₅₀ values for the α -pinene against *D. maculatus* larvae were 126.34, 57.94 and 14.68 $\mu\text{L}/\text{cm}^3$, and 187.29, 79.43 and 37.53 $\mu\text{L}/\text{cm}^3$ against *D. maculatus* adults at the exposure of 24, 48 and 72 h respectively (Table 2, Fig. 6). The results also varied significantly ($P < 0.001$) and there was no overlapping at 95% CL. Eucalyptus oil were more effective than α -pinene against larvae and adults of

D. maculatus.

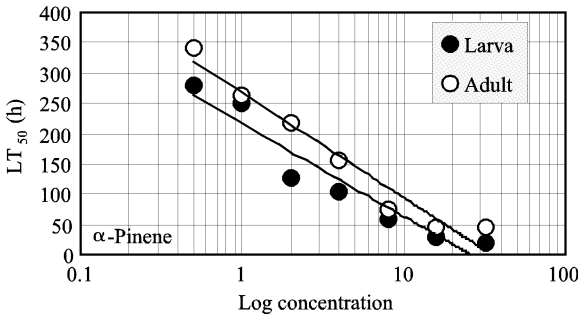


Fig. 6 Probit mortality for lethal time of *Dermestes maculatus* larvae and adults fumigated with α -pinene at different concentrations

Table 2 Probit mortality for the α -pinene against *Dermestes maculatus* fumigated at different time intervals

Developmental stage of insect	Exposure (h)	LC ₅₀ ($\mu\text{L}/\text{cm}^3$)	Lower – Upper (95% CL)	Slope \pm SE	χ^2	Probability
Larva	24	126.34	48.68 – 176.67	0.81 \pm 0.12	2.50	0.51
	48	57.94	24.48 – 87.54	0.12 \pm 0.10	5.21	0.61
	72	14.68	8.97 – 48.94	1.15 \pm 0.17	2.52	0.69
Adult	24	187.29	104.18 – 184.67	0.42 \pm 0.14	0.81	0.42
	48	79.43	57.39 – 107.67	0.72 \pm 0.13	3.74	1.92
	72	37.53	19.57 – 75.37	0.69 \pm 0.13	2.59	1.47

4 DISCUSSION

The above results showed that the phytochemicals were found to be highly toxic against the larvae of *D. maculatus* at exposure of 72 h, indicating the lower values of LT₅₀ and LC₅₀ than the adults. The highest mortality for *D. maculatus* larvae was recorded at the dose level of 32 $\mu\text{L}/\text{cm}^3$, while the lowest at 0.5 $\mu\text{L}/\text{cm}^3$. These results are in agreement with the findings of Don-Pedro (1989), who worked with some vegetables oils against *D. maculatus* Deg. (Coleoptera: Dermestidae). Laboratory studies have also been conducted on the efficacy of vegetables oils such as groundnut, coconut, palm, corn, sunflower and sesame for protecting dried fish against insects (Okorie *et al.*, 1990; Yu, 1994; Rajendran and Hajira Parveen, 2005). Unlike vegetable oils, citrus peel oils showed activity against the pests for a short period of ≤ 24 h only (Don-Pedro, 1996). The present results support the findings of Fasakin and Aberejo (2002), who reported that the pulverised plant materials including *T. diversifolia*, *A. melegueta*, *N. tabacum*, *M. myristica* and *P. guineense*, cause 100% mortality for *D. maculatus* larvae, and also mentioned that larval stages of *D. maculatus* were

more susceptible to the powders of plant materials than adult stages. Similar results have been reported for the toxicity of β -asarone to adults of *S. oryzae* and *L. serricorne* (Park, 2000). However, the adulticidal activity of eugenol against *S. oryzae* and *L. serricorne* is dependent upon both dose and exposure time (Ha, 2000). In our study, the insecticidal activity of the plant-derived compounds varied according to both dose and exposure time. The insecticidal action of eugenol against *D. maculatus* adults was very rapid. The vapor activity of oregano, perilla, tea tree, lavender, clove, and geranium oils against *Trichophyton mentagrophytes* in a closed box (Inouye *et al.*, 2006). Tripathi *et al.* (2002) reported that bioactivities of the leaf essential oil of *Curcuma longa* (var. *ch-66*) on three species of stored-product beetles (Coleoptera). In our study, eucalyptus oil and α -pinene were much more effective against *D. maculatus* larvae in the closed fumigation chamber. These results indicate that the insecticidal mode of action of the compounds may be largely penetrating the insect body *via* the respiratory system. El-Nahal *et al.* (1989) stated that the exposure period appeared to be the more important factor affecting the toxic effects of the vapors *Acorus calamus* L. essential oil on adults of five stored-product insect species rather than the

dose. Similar results have been reported for the fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Chaubey, 2008). However, the adulticidal activity of (E)-anethole, fenchone and estragole against *S. oryzae*, *C. chinensis* and *L. serricornis* is dependent upon both dose and exposure time (Kim and Ahn, 2001). The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Sim *et al.*, 2006; Kim *et al.*, 2007). Due to their high volatility, they have fumigant action and gaseous action might be of importance for stored-product insects.

ACKNOWLEDGEMENTS We would like to thank Carl Roth GmbH Berlin, Germany for supplying phytochemicals for experimentation. The author is grateful to the staffs, Key Laboratory of Insect Resource Utilization & Sustainable Pest Management of Hubei Province, Huazhong Agricultural University, Wuhan, Hubei, China for kind help during the research work.

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桉树精油及其活性物质对干鱼害虫 白腹皮蠹的熏蒸毒性

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摘要: 为了评估桉树精油和 α -蒎烯对白腹皮蠹 *Dermestes maculatus* Degeer 的杀虫作用, 采用生测方法测定了不同剂量和不同处理时间下, 其幼虫和成虫的死亡率。结果表明: 用桉树精油及其活性成分 α -蒎烯处理白腹皮蠹成虫和幼虫后, 可显著影响其死亡率 ($P < 0.001$)。白腹皮蠹的反应因化学物种类、剂量和暴露时间不同而异。当剂量为 $32 \mu\text{L}/\text{cm}^3$ 的桉树精油处理白腹皮蠹幼虫 72 h 后, 死亡率可超过 90%。同样的剂量对其成虫熏蒸 72 h 后也表现高毒。在相同的剂量和相同的暴露时间下, α -蒎烯对白腹皮蠹的成虫和幼虫也表现高毒, 且成虫的抗性比幼虫强。Probit 检验结果表明桉树精油比 α -蒎烯更有效。以上结果说明这些天然产物对白腹皮蠹种群的控制是很有用的。

关键词: 桉树精油; α -蒎烯; 熏蒸; 成虫; 幼虫; 干鱼

中图分类号: Q965.9 文献标识码: A 文章编号: 0454-6296(2009)01-110-06

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